TRANSCRIPTOME ANALYSIS BY MICROARRAY

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Organisms have to continuously adjust to changing environmental conditions and their programs of growth and development. Much of the biological regulations arising from these functions occur at the level of transcriptional control of genes involved in these processes. Thus comprehensive knowledge of the genes that change their expression pattern in response to a signal may reveal much on the mechanisms that are turned on.

The **transcriptome** is the set of all RNA molecules, including mRNA, rRNA, tRNA, and other non-coding RNA produced in one or a population of cells. Globally transcriptome analysis is of growing importance in understanding how altered expression of genetic variants contributes to complex diseases such as cancer, diabetes and cardiac disease. Analysis of differential expression of genes in particular population of cells provides researchers with greater insights into biological pathways and molecular mechanisms that regulate varied aspects of cell fate, development and disease progression.

SCOPE

The term transcriptome can be applied to the total set of RNA transcripts in a given organism, or to the specific subset of transcripts present in a particular cell type. Unlike the genome, which is roughly fixed for a given cell line (excluding mutations which are rare), the transcriptome can vary with environmental and experimental conditions, diet etc. Because it includes all *mRNA* transcripts in the cell, the transcriptome reflects the genes that are being actively expressed at any given time,. The study of *transcriptomics*, also referred to as expression profiling, examines the expression level of mRNAs in a given cell population, often using techniques based on DNA microarray technology.

APPLICATIONS

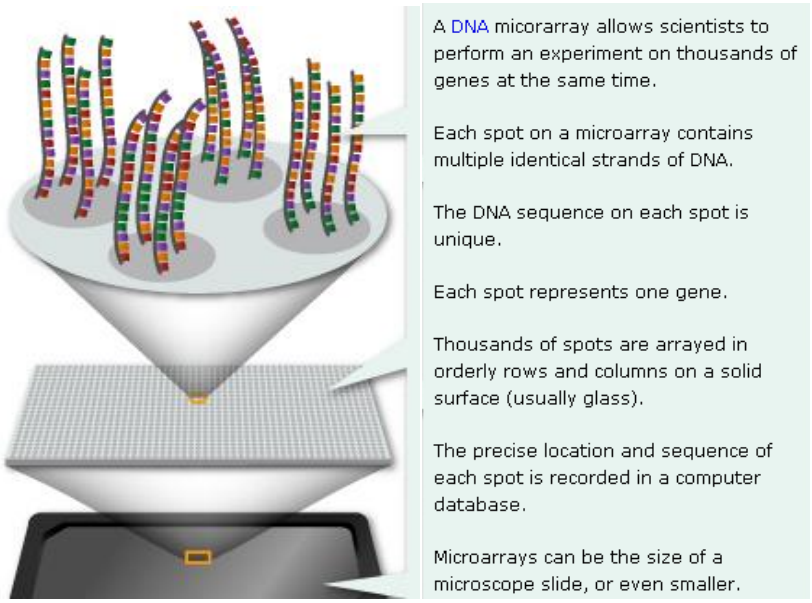
Transcriptome analysis has wide applications, particularly in medical biology. The transcriptomes of stem cells and cancer cells are of particular interest to researchers who seek to understand the processes of cellular differentiation and carcinogenesis. Analysis of the transcriptomes of human oocytes and embryos is used to understand the molecular mechanisms and signaling pathways controlling early embryonic development, and could theoretically be a powerful tool in making proper embryo selection in *in vitro* fertilization. The method may also be highly applicable to understanding change in gene expression profile in view of change in environment including pollution and climate change and phonological events.

DNA MICROARRAY

A **microarray** is a set of short **Expressed Sequence Tags** (ESTs) made from a **cDNA** library of a set of known (or partially known) gene loci. The **ESTs** are spotted onto a cover-slip-sized glass plate. In practice, microarrays of many thousand **ESTs** are possible.

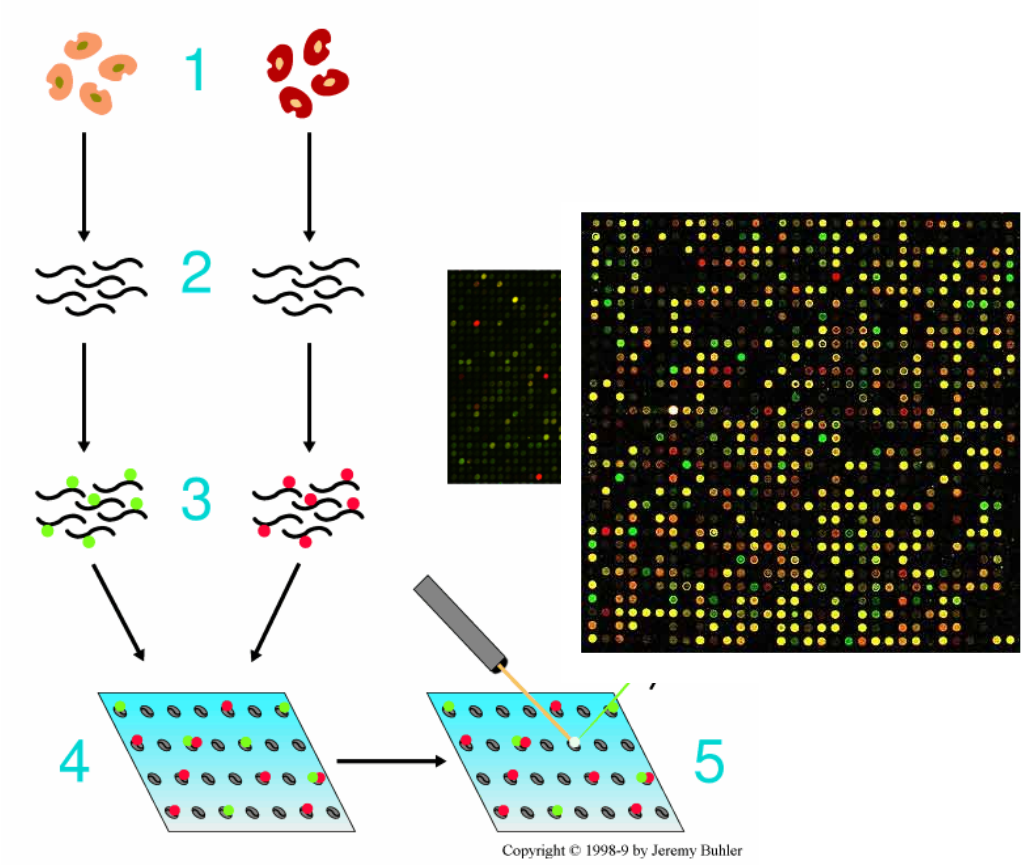
The major steps of a comparative cDNA hybridization experiment are:

- Choosing Cell Populations under observation and control
- Isolation of transcriptome & mRNA extraction from both by means of an oligo-dT column
- Reverse transcription to prepare the cDNAs
- Fluorescent Labeling of cDNAs
- Hybridization to a DNA Microarray
- Dual channel LASER excitation
- Scanning the Hybridized Array and Interpretation of the Scanned Image



PROCEDURE OF MICROARRAY

- First, the transcripts or RNA molecules from a experimental cell or population of cells are isolated.
- cDNA is made from them by reverse transcriptase. These are test cDNAs.
- This test cDNA collection or library is kept for analysis.
- The microarray is prepared by spotting oligonucleotide probes corresponding to genes under observation on glass slides by a robotic spotter.
- The microarray can be also be prepared by *in situ* synthesis of oligonucleotide probes.
- Now the test cDNAs are labeled with red flourophores and added to the microarray.
- Similarly control cDNAs are also prepared, labeled with a different (*e.g.*green) flourophore and added to the microarray.
- Hybridisation occurs wherever there occurs sequence complementarity.
- The flourophores are excited by dual LASER (light of single wave length) for two florophores.
- On excitation, the fluorophores emit light.
- These are photographically recorded and analysed.
- In the photograph, red spots correspond to genes which express more or exclusively in test and are said to be **up-regulated**.
- On the other hand, green spots correspond to genes which express less or not at all in test (*i.e.* more or exclusively in control) and are said to be **down-regulated**.
- Yellow spots correspond to genes which are equally expressed in both test and control.
- Black spots correspond to no expression of the corresponding gene in either test or control.
- The entire analysis of the signal and its intensity is by computer.
- Accordingly, we can understand the extent of expression of huge number of genes at a time in test and control and measure the difference between the two.



USES OF MICROARRAY

The method can be applied to test difference in gene expression pattern in:

a) Different tissues or cells
b) Diseases like cancer, cardiac disease, diabetes etc.
c) Cells or organisms exposed to pollutants
d) Cells or organisms receiving particular drug(s) and thus can be useful in drug designing
e) Organisms infected by parasitic organisms
f) Embryonic developmental stages etc.